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Behavioural studies of the actions of cocaine, monoamine oxidase inhibitors and iminodibenzyl compounds on central dopamine neurones

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Summary

1. Turning behaviour after unilateral lesions of the nigro-striatal dopamine pathway in rats has been used to compare the actions of cocaine and desipramine on central dopamine-containing neurones.

2. Administration of cocaine alone (5–20 mg/kg) resulted in no turning or minimal turning towards the lesioned side; the monoamine oxidase inhibitors nialamide and pargyline administered alone were also ineffective.

3. After pre-treatment with nialamide (100 mg/kg) or pargyline (25 mg/kg) cocaine evoked high rates of turning towards the lesioned side.

4. Desipramine (1–100 mg/kg), either alone or in combination with nialamide did not evoke turning.

5. Turning evoked by the cocaine-nialamide combination was abolished by pre-treatment with α-methyl-*p*-tyrosine (150 mg/kg).

6. Pre-treatment with reserpine (5 mg/kg, 24 h previously) substantially diminished turning evoked by the cocaine-nialamide combination but potentiated turning resulting from administration of methylamphetamine (5 mg/kg).

7. Cocaine (20 mg/kg) administered 15 min prior to (+)-methylamphetamine (5 mg/kg) reduced the turning behaviour in the first hour after administration of the latter drug but prolonged the total duration of the effect.

Introduction

Turning behaviour induced by various drugs in rats with unilateral lesions of the uncrossed nigro-striatal dopamine pathway (Andén, 1966) can be used as an index of the action of such drugs on central dopamine-containing neurones (Ungerstedt & Arbuthnott, 1970; Christie & Crow, 1971; Ungerstedt, 1971). This turning behaviour probably reflects an increased release of dopamine from terminals of the nigro-striatal pathway on the intact side since (1) turning occurs only in animals with lesions which interrupt the nigro-striatal pathway (Crow, 1971), (2) such lesions are associated with loss in the ipsilateral corpus striatum of the fluorescence due to the presence of dopamine (Arbuthnott & Crow, 1971), (3) turning following amphetamine and ephedrine administration is abolished (Christie & Crow, 1971) by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine, but not by the drug bis-(4-methyl-1-homopiperazinylthiocarbonyl)-disulphide (FLA63) which is a dopamine-β-oxidase inhibitor (Svensson & Waldeck, 1969). Turning can also be induced by amphetamine in rats with nigro-striatal lesions produced by localized intracerebral injections of 6-hydroxydopamine (Ungerstedt,

1971), and by electrical stimulation in the region of the nigro-striatal pathway (Arbuthnott, Crow, Fuxe & Ungerstedt, 1970; Arbuthnott & Crow, 1971).

Experiments on the inhibition of uptake of tritiated catecholamines into cortical and striatal slices (Ross & Renyi, 1967; Fuxe, Hamberger & Malmfors, 1967) suggest that there are differences between the neuronal uptake mechanisms for noradrenaline and for dopamine. Desipramine inhibits noradrenaline uptake into cortical slices at a concentration of 3×10^{-8}M, but inhibits uptake of dopamine into striatal slices only at a concentration of 5×10^{-5}M. Cocaine, however, inhibits cortical noradrenaline uptake at a concentration of 1×10^{-6}M and striatal dopamine uptake at the similar concentration of 2×10^{-6}M (Ross & Renyi, 1967). Thus the relative desipramine:cocaine potencies are 33:1 in blocking noradrenaline uptake, and 1:25 in blocking dopamine uptake.

In the present experiments, turning behaviour has been used to investigate and compare the *in vivo* interactions between cocaine, desipramine and dopamine-containing neurones.

Methods

Male hooded Lister rats, weighing 200 ± 20 g, were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg body weight). Unilateral lesions in the substantia nigra were made by passing a charge of 40 mC through a varnished steel electrode (anode) implanted stereotaxically (co-ordinates according to the atlas of Fifkova & Marsala (1967): P4·5 L1·0 V8·5), the circuit being completed by an anal cathode.

At least 1 week after recovery from the operation, the rats were tested for turning towards the lesioned side after an intraperitoneal injection of (+)-methylamphetamine (5 mg/kg). Forty rats which showed more than 10 turns/min after (+)-methylamphetamine were selected for further drug trials. These rats were divided into five groups of eight, and direct comparisons were made between rats within these groups. At least six weeks were allowed between each drug trial. The number of turns towards the side of the lesion in one minute was recorded at 15 min intervals for each rat.

The following drugs were used: (+)-amphetamine sulphate (Smith, Kline & French); (+)-methylamphetamine hydrochloride (Burroughs Wellcome); α-methyl-*p*-tyrosine methylester hydrochloride (Axel Kistner AB); cocaine hydrochloride (Macfarlan Smith); bis-(4-methyl-1-homopiperazinylthiocarbonyl)-disulphide (FLA63) (AB Biotec); desipramine hydrochloride (Geigy); nialamide hydrochloride (Pfizer); pargyline hydrochloride (Abbott); reserpine (B.D.H.). All drugs were dissolved in 0·9% w/v NaCl solution with the exceptions of reserpine, which was dissolved in the minimum amount of glacial acetic acid and diluted with distilled water, and FLA63, which was dissolved in the minimum amount of 1 N HCl, diluted with 0·9% w/v NaCl and brought to pH 6 with 0·1 N NaOH. All drugs were administered by intraperitoneal injection. Doses of the drugs given refer to the weights of the salt.

Results

Cocaine

Cocaine in doses of 5 to 20 mg/kg evoked only minimal turning (i.e. 3 turns/min at the highest dose) (Fig. 1). The monoamine oxidase (MAO) inhibitor nialamide

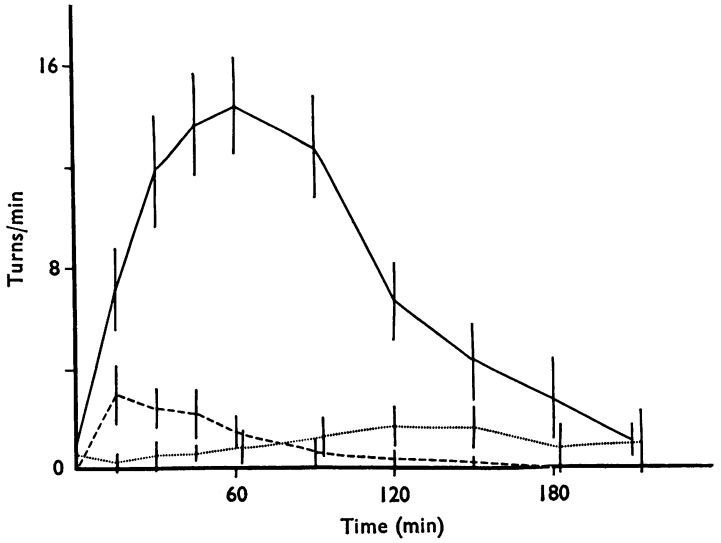

FIG. 1. Turning behaviour in rats after cocaine 20 mg/kg (- - - -), nialamide 100 mg/kg (· · · · ·), and after cocaine 20 mg/kg, 2 h after nialamide 100 mg/kg (——). Bars ± 1 S.E.M.

(100 mg/kg) was also ineffective (Fig. 1). The combination, however, i.e. cocaine (20 mg/kg) 2 h after nialamide (100 mg/kg) evoked a high rate of turning (Fig. 1).

Similar results were obtained with the MAO inhibitor pargyline. Pargyline (25 mg/kg) itself produced no turning. However, cocaine (20 mg/kg), given 2 h after pargyline (25 mg/kg) evoked a mean maximum turning rate of 27 turns/min, 30 min after cocaine administration. The time-course of turning in this case was closely similar to that seen after the cocaine-nialamide combination (Fig. 1). Twelve hours after pargyline (25 mg/kg), cocaine (20 mg/kg) induced severe toxic symptoms (tremor and pyrexia progressing to convulsions and death) but a smaller dose of cocaine (10 mg/kg) evoked turning of similar magnitude and time-course to that seen after the cocaine-nialamide combination.

Desipramine

Desipramine (1–100 mg/kg) did not evoke turning and no interaction between desipramine and nialamide occurred.

Pre-treatment with inhibitors of catecholamine synthesis

Turning evoked by the combination of cocaine (20 mg/kg) 2 h after nialamide (100 mg/kg) was almost abolished by pre-treatment with the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (150 mg/kg, 12 h before the injection of cocaine (Fig. 2)). When the cocaine-nialamide combination was given after administration of the dopamine-β-oxidase inhibitor FLA63 (50 mg/kg 2 h before the nialamide) severe ataxia and limb hyperextension were induced. After this combination of treatments animals showed lateral flexion towards the lesioned side. They also fell frequently towards the non-lesioned side, but did not show turning.

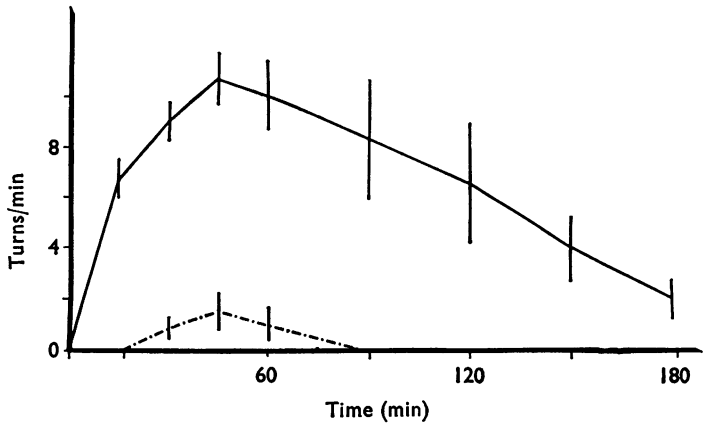

FIG. 2. Turning behaviour after cocaine 20 mg/kg, 2 h after nialamide 100 mg/kg (——), and after cocaine 20 mg/kg and nialamide 100 mg/kg after the rats had been treated 12 h earlier with α-methyl-*p*-tyrosine 150 mg/kg (- · - · -). Bars ± 1 S.E.M.

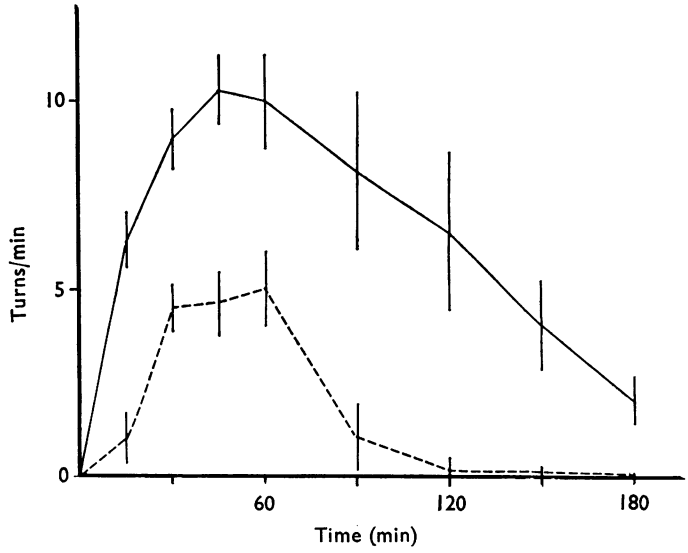

FIG. 3. Turning behaviour after cocaine 20 mg/kg, 2 h after nialamide 100 mg/kg when given alone (——) and when given 24 h after reserpine 5 mg/kg (- - -). Bars ± 1 S.E.M.

Pre-treatment with reserpine

Reserpine (5 mg/kg) 22 h prior to nialamide (100 mg/kg) and 24 h before cocaine (20 mg/kg) greatly reduced the turning evoked by the cocaine-nialamide combination (Fig. 3).

Pre-treatment with reserpine (5 mg/kg) 24 h before (+)-amphetamine (5 mg/kg) significantly potentiated the amphetamine-induced turning (Fig. 4).

Cocaine-methylamphetamine interaction

Administration of cocaine (20 mg/kg) 15 min before (+)-methylamphetamine (5 mg/kg) reduced the turning produced in the first 60 to 90 min after (+)-methyl-

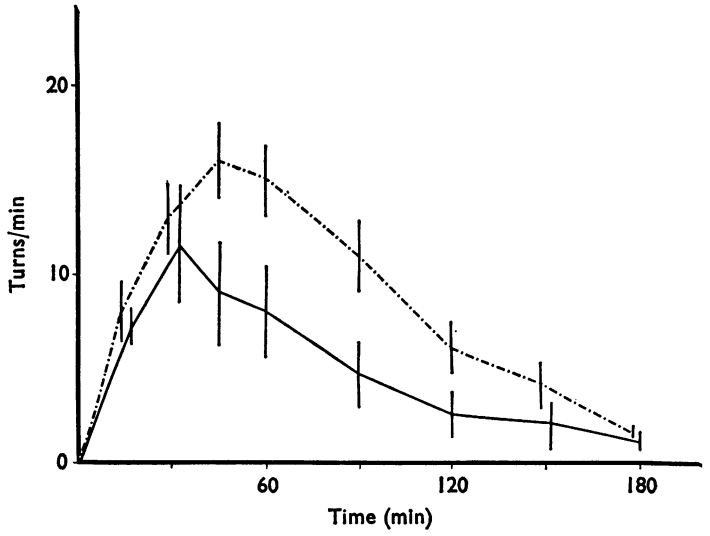

FIG. 4. Turning behaviour after amphetamine 5 mg/kg when given alone (——) and when given 24 h after reserpine 5 mg/kg (- · · · -). Bars ± 1 S.E.M.

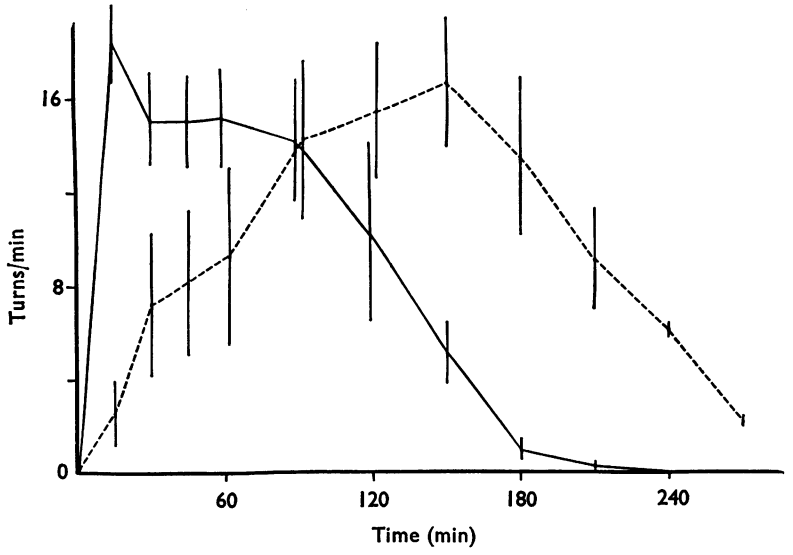

FIG. 5. Turning behaviour after methylamphetamine 5 mg/kg when given alone (——) and when given 15 min after cocaine 20 mg/kg (- - - - -). Bars ± 1 S.E.M.

amphetamine but prolonged the duration of the turning (Fig. 5). A smaller dose of cocaine (10 mg/kg) prolonged the duration of turning without effecting an initial inhibition (Fig. 6).

Desipramine-methylamphetamine interaction

Desipramine (20–100 mg/kg) 15 or 30 min before (+)-methylamphetamine (5 mg/kg) did not inhibit turning but a dose of 20 mg/kg greatly prolonged the amphetamine response (Fig. 7). The higher doses of desipramine generally proved lethal.

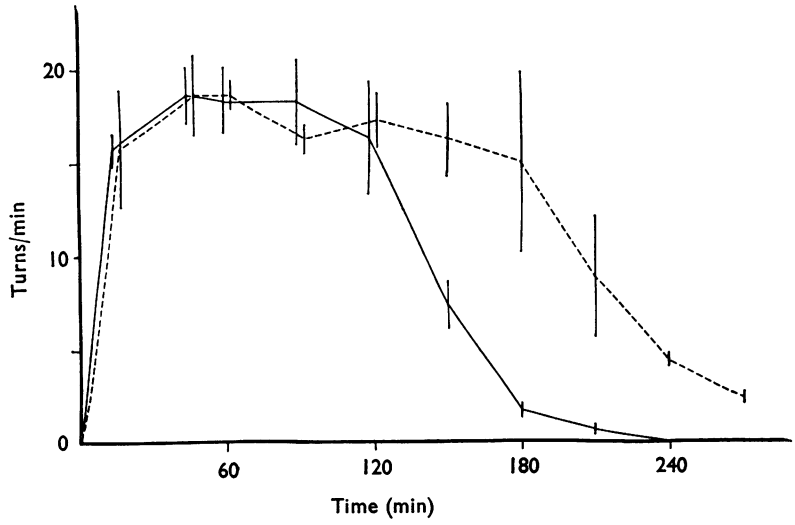

FIG. 6. Turning behaviour after methylamphetamine 5 mg/kg when given alone (——) and when given 15 min after cocaine 10 mg/kg (- - - -). Bars ± 1 S.E.M.

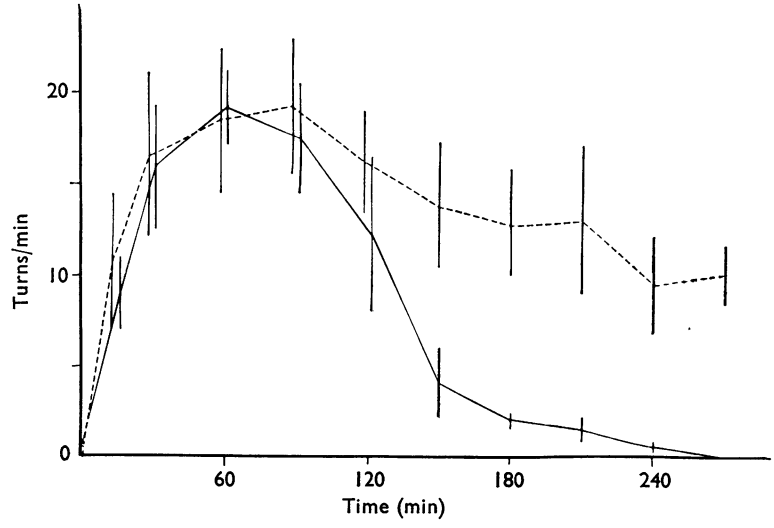

FIG. 7. Turning behaviour after methylamphetamine 5 mg/kg when given alone (——) and when given 15 min after desipramine 20 mg/kg (- - - -). Bars ± 1 S.E.M.

Discussion

Turning behaviour is observed after administration of cocaine and a MAO inhibitor (nialamide or pargyline). That this turning is abolished by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine, is consistent with the hypothesis that turning reflects increased release of cerebral catecholamine stores. However, neither administration of cocaine, which blocks the neuronal mechanism for dopamine uptake (Ross & Renyi, 1967) nor of a MAO inhibitor, which increases cerebral monoamine concentrations (Everett & Wiegand, 1962), separately, will cause turning behaviour. A possible interpretation of the interaction therefore is that turning

occurs only when there is an increase in cerebral dopamine concentration combined with inhibition of the neuronal uptake process. However, the importance of uptake blockade for potentiation of responses to catecholamines by cocaine has recently been questioned (Maxwell, Wastila & Eckhardt, 1966), and other possibilities, e.g. receptor sensitization (Reiffenstein, 1968 ; Varma & McCullough, 1969 ; Kalsner & Nickerson, 1969) have been suggested.

There are several possible modes of interaction between the amphetamines and dopamine-containing neurones—(i) blockade of dopamine uptake (Ross & Renyi, 1967) ; (ii) MAO inhibition (Blaschko, Richter & Schlossmann, 1937 ; Glowinski, 1970), and (iii) release of dopamine from nerve terminals (Carlsson, 1970 ; Fuxe & Ungerstedt, 1970). Since neither cocaine alone, nor nialamide or pargyline alone causes turning, it seems unlikely that the action of the amphetamines is mediated solely by either mechanism (i) or (ii). Since the combination of cocaine and a MAO inhibitor is as effective as amphetamine in provoking turning, one must consider whether the amphetamines act by a combination of actions (i) and (ii) (i.e. uptake blockade *and* MAO inhibition). However, the amphetamines are weak inhibitors of the dopamine uptake process by comparison with cocaine (Ross & Renyi, 1967). Moreover, reserpine (5 mg/kg) given 24 h previously greatly diminishes the action of the cocaine–MAO inhibitor combination but potentiates that of (+)-amphetamine. These interactions suggest that the cocaine–MAO inhibitor combination and the amphetamines cause dopamine release by dissimilar mechanisms.

Cocaine (20 mg/kg) administered 15 min before (+)-methylamphetamine inhibits the turning produced by the latter drug. A similar antagonism of the action of the amphetamines by cocaine has been described in several studies of the sympathetic postganglionic neurone (Fleckenstein & Stöckle, 1955 ; Trendelenburg, Muskus, Fleming & Gomez, 1962 ; Trendelenburg, 1963). The duration of turning induced by methylamphetamine is, however, prolonged by the administration of cocaine. This may be a metabolic effect since cocaine reduces *p*-hydroxylation of amphetamines in the liver (Lewander, 1969).

Desipramine (20–100 mg/kg) does not diminish turning behaviour in the first hour after methylamphetamine 5 mg/kg, and therefore lacks this action of cocaine on central dopamine neurones. This difference between the iminodibenzyl compounds and cocaine is probably in contrast to the situation at the peripheral adrenergic neurone where both imipramine and cocaine can antagonize the sympathetic effects of the amphetamines (Ryall, 1961). Desipramine does, however, prolong the duration of turning as does protriptyline (Crow & Gillbe, 1970). This effect may be due to the delayed fall in tissue concentrations of amphetamines which follows pre-treatment with iminodibenzyl compounds (Sulser, Owens & Dingell, 1966 ; Consolo, Dolfini, Garattini & Valzelli, 1967 ; Dolfini, Tansella, Valzelli & Garattini, 1969 ; Lewander, 1969).

The results of these experiments provide behavioural evidence for an action of cocaine on central dopamine-containing neurones. The interactions of cocaine with the amphetamines and MAO inhibitors are consistent with the hypothesis that cocaine blocks the neuronal uptake process. The tricyclic antidepressant compounds do not share this action and this may account for the differences between the actions of this group of compounds and cocaine on exploratory activity, since

administration of cocaine causes an increase in locomotor activity (van Rossum, van der Schoot & Hurkmans, 1962), while the tricyclic antidepressants decrease total activity (Herr, Stewart & Charest, 1961).

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